TABLE I3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.22 ± 0.72	21.1–23.5	13
Crude fat (% by weight)	5.59 ± 0.55	4.7–6.4	13
Crude fiber (% by weight)	3.36 ± 0.30	2.7–3.8	13
Ash (% by weight)	6.55 ± 0.23	6.1–7.0	13
Amino Acids (% of total diet)			
Arginine	1.308 ± 0.606	1.210–1.390	8
Cystine	0.306 ± 0.084	0.181–0.400	8
Glycine	1.150 ± 0.047	1.060–1.210	8
Histidine	0.576 ± 0.024	0.531–0.607	8
Isoleucine	0.917 ± 0.029	0.881–0.944	8
Leucine	1.946 ± 0.055	1.850–2.040	8
Lysine	1.270 ± 0.058	1.200–1.370	8
Methionine	0.448 ± 0.128	0.306–0.699	8
Phenylalinine	0.987 ± 0.140	0.665–1.110	8
Threonine	0.877 ± 0.042	0.824–0.940	8
Tryptophane	0.236 ± 0.176	0.107–0.671	8
Tyrosine	0.676 ± 0.105	0.564–0.794	8
Valine	1.103 ± 0.040	1.050–1.170	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.393 ± 0.258	1.830–2.570	7
Linolenic	0.280 ± 0.040	0.210–0.320	7
Vitamins			
Vitamin A (IU/kg)	9,846 ± 2,839	5,600–15,000	13
Vitamin D (IU/kg)	4,450 ± 1,382	3,000–6,300	4
e-Tocopherol (ppm)	37.95 ± 9.41	22.5–48.9	8
Thiamine (ppm)	20.77 ± 2.01	17.0–23.0	13
Riboflavin (ppm)	7.92 ± 0.87	6.10–9.00	8
Niacin (ppm)	103.4 ± 26.59	65.0–150.0	8
Pantothenic acid (ppm)	29.54 ± 3.60	23.0–34.0	8
Pyridoxine (ppm)	9.55 ± 3.48	5.60–14.0	8
Folic acid (ppm)	2.25 ± 0.73	1.80–3.70	8
Biotin (ppm)	0.254 ± 0.042	0.19–0.32	8
Vitamin B_{12} (ppb)	38.45 ± 22.01	10.6–65.0	8
Choline (ppm)	3,089 ± 328.69	2,400–3,430	8
Minerals			
Calcium (%)	1.17 ± 0.09	1.06–1.41	13
Phosphorus (%)	0.92 ± 0.03	0.87–0.99	13
Potassium (%)	0.883 ± 0.078	0.772–0.971	6
Chloride (%)	0.526 ± 0.092	0.380–0.635	8
Sodium (%)	0.313 ± 0.390	0.258–0.371	8
Magnesium (%)	0.168 ± 0.010	0.151–0.181	8
Sulfur (%)	0.280 ± 0.064	0.208–0.420	8
Iron (ppm)	360.5 ± 100	255.0–523.0	8
Manganese (ppm)	92.0 ± 6.01	81.70–99.40	8
Zinc (ppm)	54.72 ± 5.67	46.10–64.50	8
Copper (ppm)	11.06 ± 2.50	8.090–15.39	8
Iodine (ppm)	3.37 ± 0.92	1.52–4.13	6
Chromium (ppm)	1.79 ± 0.36	1.04–2.09	8
Cobalt (ppm)	0.681 ± 0.14	0.490–0.780	4

TABLE I4
Contaminant Levels in NIH-07 Rat and Mouse Ration

	Mean ± Standard Deviation[a]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.72 ± 0.19	0.33–0.94	13
Cadmium (ppm)	<0.1		13
Lead (ppm)	0.57 ± 0.31	0.14–1.32	13
Mercury (ppm)	<0.05		13
Selenium (ppm)	0.35 ± 0.08	0.21–0.44	13
Aflatoxins (ppb)	<5.0		13
Nitrate nitrogen (ppm)[b]	12.56 ± 4.47	2.80–18.0	13
Nitrite nitrogen (ppm)[b]	0.14 ± 0.11	<0.10–0.50	13
BHA (ppm)[c]	2.54 ± 1.05	<2.00–5.00	13
BHT (ppm)[c]	2.39 ± 1.33	<1.00–4.00	13
Aerobic plate count (CFU/g)[d]	39,523 ± 39,878	3,400–130,000	13
Coliform (MPN/g)[e]	3.72 ± 1.79	<3.00–9.00	11
Coliform (MPN/g)[f]	9.46 ± 14.11	<3.00–43.0	13
E. coli (MPN/g)[g]	3.08 ± 0.28	<3.0–4.00	13
Total nitrosamines (ppb)[h]	6.99 ± 4.13	1.80–16.00	13
N-Nitrosodimethylamine (ppb)[h]	5.67 ± 3.79	0.80–15.00	13
N-Nitrosopyrrolidine (ppb)[h]	1.32 ± 0.73	1.00–3.40	13
Pesticides (ppm)			
α-BHC[i]	<0.01		13
β-BHC	<0.02		13
γ-BHC	<0.01		13
δ-BHC	<0.01		13
Heptachlor	<0.01		13
Aldrin	<0.01		13
Heptachlor epoxide	<0.01		13
DDE	<0.01		13
DDD	<0.01		13
DDT	<0.01		13
HCB	<0.01		13
Mirex	<0.01		13
Methoxychlor	<0.05		13
Dieldrin	<0.01		13
Endrin	<0.01		13
Telodrin	<0.01		13
Chlordane	<0.05		13
Toxaphene	<0.1		13
Estimated PCBs	<0.2		13
Ronnel	<0.01		13
Ethion	<0.02		13
Trithion	<0.05		13
Diazinon	<0.1		13
Methyl parathion	<0.02		13
Ethyl parathion	<0.02		13
Malathion[j]	0.09 ± 0.07	0.05–0.28	13
Endosulfan I	<0.01		13
Endosulfan II	<0.01		13
Endosulfan sulfate	<0.03		13

TABLE I4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Sources of contamination: alfalfa, grains, and fish meal
[c] Sources of contamination: soy oil and fish meal
[d] CFU = colony forming unit
[e] MPN = most probable number
[f] Includes two high values of 39 and 43 MPN/g obtained from lots milled 15 March 1984 and 9 May 1984, respectively.
[g] One lot milled 17 October 1984 contained 4.00 MPN/g; all other lots contained 3.00 MPN/g
[h] All values were corrected for percent recovery.
[i] BHC = hexachlorocyclohexane or benzene hexachloride.
[j] Seven lots contained more than 0.05 ppm.

APPENDIX J
SENTINEL ANIMAL PROGRAM

METHODS J-2
TABLE J1 Murine Virus Antibody Determinations for Rats and Mice in the Lifetime and 2-Year Inhalation Studies of Talc J-4

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Rats

Prior to the beginning of the lifetime study, 5 F344/N rats of each sex were sacrificed and serum samples were taken for serological evaluation by Microbiological Associates (Bethesda, MD). Serum samples were also taken from selected rats for serology testing at each of the interim evaluations: 3 male and 3 female rats at 6 months; 8 male and 9 female rats at 12 and 18 months; 11 male and 17 female rats at 24 months; and 15 male and 15 female rats at the terminal sacrifice (male, 113 weeks; female, 122 weeks). Blood collected from each animal was allowed to clot and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates (Bethesda, MD) for determination of antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
ELISA	
RCV/SDA (rat corona virus/sialodacryoadenitis virus)	Study initiation, 6, 12, 18, 24 months, study termination
PVM (pneumonia virus of mice)	6, 12, 18, 24 months, study termination
Sendai	6, 12, 18, 24 months, study termination
Mycoplasma arthritidis	12, 18, 24 months, study termination
Mycoplasma pulmonis	12, 18, 24 months, study termination
CARB (cilia-associated respiratory bacillus)	Study termination (males only)
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	Study initiation, 6, 12, 18, 24 months, study termination
KRV (Kilham rat virus)	Study initiation, 6, 12, 18, 24, study termination
PVM	Study initiation
Sendai	Study initiation
Immunofluorescence Assay	
KRV	24 months (males only)
RCV (rat corona virus)	24 months (males only)
RCV/SDA	28 months (males only)

Mice

Prior to the beginning of the 2-year study, 5 B6C3F$_1$ mice of each sex were sacrificed and serum samples were taken for serological evaluation by Microbiological Associates (Bethesda, MD). Serum samples for serology testing were also taken from control males and females at each of the interim evaluations (4 males and 4 females at 6 months; 12 males and 12 females at 12 months) and at the terminal sacrifice (15 males and 15 females). (Samples were inadvertently omitted for mice evaluated after 18 months of exposure on 4-5 December, 1985.) Blood collected from each animal was allowed to clot and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates (Bethesda, MD) for determination of antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Complement Fixation	
LCM (lymphocytic choriomeningitis virus)	Study initiation, 6, 12, 24 months
Mouse adenoma virus	Study initiation
ELISA	
Ectromelia virus	6, 12, 24 months
GDVII (mouse encephalomyelitis virus)	Study initiation, 6, 12, 24 months
MHV (mouse hepatitis virus)	Study initiation, 6, 12, 24 months
PVM	6, 12, 24 months
Sendai	6, 12, 24 months
Reo 3	6, 12, 24 months
Mouse adenoma virus	6, 12, 24 months
M. arthritidis	6, 12, 24 months
M. pulmonis	6, 12, 24 months
Hemagglutination Inhibition	
Ectromelia virus	Study initiation
K (papovirus)	12, 24 months
MVM (minute virus mice)	Study initiation, 6, 12, 24 months
PVM	Study initiation
Polyoma virus	Study initiation, 6, 12, 24 months
Reovirus 3	Study intitiation
Sendai	Study initiation
Immunofluorescence Assay	
EDIM (Epizootic diarrhea of infant mice)	6, 12, 24 months
Reovirus 3	24 months

TABLE J1
Murine Virus Antibody Determinations for Rats and Mice in the Lifetime and 2-Year Inhalation Studies of Talc

Interval (months)	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
Rats		
6 months	0/6	–
12 months	0/17	–
18 months	0/17	–
24 months		
(males)	1/11	KRV
	9/11	Sendai
	6/11	RCV
(females)	13/17	Sendai
	13/17	RCV/SDA
28 months		
	15/15	Sendai
	3/15	RCV/SDA
30 months	15/15	Sendai
	1/15	RCV/SDA
Mice		
6 months	0/8	–
12 months	0/24	MHV
24 months	2/30	Reovirus 3
	7/30	*M. arthritidis*
	21/30	EDIM

APPENDIX K
4-WEEK INHALATION STUDIES IN RATS AND MICE

EXPERIMENTAL PROTOCOL K-2
TABLE K1 Experimental Design and Materials and Methods in the 4-Week Inhalation Studies of Talc K-3
RESULTS K-5

EXPERIMENTAL PROTOCOL

Procurement and Characterization of Talc

Talc was obtained from Walsh and Associates (North Kansas City, MO) in one lot (lot number W101882). Identity and purity analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO).

The study chemical, a finely powdered white solid, was identified as talc by infrared spectroscopy, elemental analysis, and microscopic analyses. The moisture content of the bulk chemical was analyzed and was determined to be stable throughout the studies. Bulk chemical studies were not conducted due to the physical and chemical properties of talc. The compound was stored in sealed Nalgene containers.

Generation and Monitoring of Chamber Concentrations

Talc aerosols were generated in a fluidized bed generator by injecting filtered air into the bed. Samples were collected continuously during the 6-hour exposure day on glass fiber filters. Only one sampling port position was used each day to collect the samples from each chamber. Once a week, samples were collected on Zefluor filters so that the magnesium content of aerosolized talc could be determined and be compared to the magnesium content of bulk talc. Cascade impactor samples were taken 3 to 6 times a week to determine aerosol particle size. The amount of talc collected on the filters and impactor stages was quantitated gravimetrically. The extent of carry over of the stainless steel material from the FBG was quantitated by measuring the amount of acid soluble nickel and chromium in filter samples taken from the exposure atmosphere twice during the study.

Study Design

Groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were exposed by inhalation to talc at target concentrations of 0 (chamber controls), 2, 6, and 18 mg/m^3. Rats and mice were exposed for 6 hours daily, 5 days a week, for 20 days.

Source and Specification of Animals

Male and female F344/N rats were obtained from Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM). Male and female B6C3F$_1$ mice were obtained from Simonsen Laboratory (Gilroy, CA). Rats and mice were held 3 weeks before the studies began, and were 6 to 7 weeks old when the studies began. Animal health was monitored by serologic analyses during the studies under the protocols of the NTP Sentinel Animal Program.

Animal Maintenance

Rats and mice were housed individually throughout the studies. Drinking water was available *ad libitum*. Further details of animal maintenance are given in Table K1.

Clinical Examinations and Pathology

All rats and mice were observed twice daily. Clinical observations and body weights were recorded at the beginning of the studies, each week, and at the end of the studies. Organ weights were recorded for the heart, right kidney, liver, and lung at the end of the studies.

A necropsy was performed on all animals. During necropsy, all organs and tissues were examined for grossly visible lesions. A complete histopathologic examination was performed on all high-exposure and control animals. Tissues for microscopic examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned to a thickness of 5 μm, and stained with hematoxylin and eosin.

TABLE K1
Experimental Design and Materials and Methods in the 4-Week Inhalation Studies of Talc

Study Laboratory
Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM)

Strain and Species
Rats: F344/N rats
Mice: $B6C3F_1$ mice

Animal Source
Rats: Lovelace Inhalation Toxicology Research Institute (Albuquerque, NM)
Mice: Simonsen Laboratory (Gilroy, CA)

Time Held Before Studies
3 weeks

Average Age When Placed on Studies
6-7 weeks

Date of First Exposure
Rats: 20 April 1983
Mice: 16 June 1983

Duration of Exposure
6 hours/day, 5 days/week for 4 weeks

Date of Last Exposure
Rats: 18 May 1983
Mice: 13 July 1983

Average Age When Killed
10 to 11 weeks

Method of Sacrifice
Intraperitoneal injection of T-61 solution

Necropsy Dates
Rats: 19-20 May 1983
Mice: 14-15 July 1983

Size of Study Groups
10 males and 10 females

Method of Animal Distribution
Randomized by weight

Animals per Cage
1

Method of Animal Identification
Ear tag and toeclip

Diet
NIH-07 Rat and Mouse Ration (Zeigler, Bros., Gardner, PA) available *ad libitum* during non-exposure periods

Maximum Storage Time for Feed
Not available

Water
Automatic Watering System (Edstrom Industries, Waterford, WI), available *ad libitum*

Cages
Stainless steel mesh cages (Hazleton, Aberdeen, MD), changed once weekly

TABLE K1
Experimental Design and Materials and Methods in the 4-Week Inhalation Studies of Talc (continued)

Chambers
Stainless steel multitiered whole-body exposure chambers (H2000 and H1000, Hazleton Systems, Aberdeen, MD) washed once weekly

Excreta Pan
Techboard untreated paper (Shepherd Specialties Paper, Inc., Kalamazoo, MI), changed twice a day

Filters
Room Air and Chamber Air High Efficiency Particulate Air (HEPA) Filter, MIL Spec MIL-F-51068C (Flanders, Washington, DC), changed as required

Animal Room Environment

Rats	Mice
Average temperature: 23° C	Average temperature: 24° C
Relative humidity: 40.3%	Relative humidity: 42%
Fluorescent light: not available	Fluorescent light: not available
Room air changes: not available	Room air changes: not available

Exposure Concentrations
0, 2, 6, and 18 mg/m^3 by inhalation

Type and Frequency of Observation
Observed twice daily; body weights and clinical findings recorded at study initiation and weekly thereafter

Necropsy
Necropsy was performed on all animals.

Histopathology
Complete histopathologic examinations performed on all high-exposure and control animals. In addition to tissue masses, gross lesions, and associated lymph nodes, tissues examined included: larynx, lung, nasal turbinates, trachea, and tracheobronchial lymph nodes.

RESULTS

Rats

All rats survived to the end of the study and there were no clinical findings related to talc exposure. The mean body weights and final mean body weights of exposed male and female rats were similar to those of the controls.

There were no significant increases in any organ-weight-to-body-weight ratios in male or female rats. The talc lung burdens increased with talc exposure level; however, the ratio of lung burden to exposure concentration was somewhat higher at the 6 and 18 mg/m^3 exposure levels. The increase in talc lung burden to exposure concentration may be because the maximum ability of the respiratory tract to clear particles was exceeded at the 6 and 18 mg/m^3 exposure levels.

There was a minimal increase in the number of intra-alveolar macrophages in the lung of male and female rats exposed to 18 mg/m^3. The lesion was diffuse in nature and in no instance were clusters of greater than three alveolar macrophages observed. The individual macrophages were slightly larger than normal and had cytoplasm which contained fine eosinophilic granules.

Mice

One male mouse exposed to 2 mg/m^3 and one male mouse exposed to 6 mg/m^3 died before the end of the study. The survival of exposed male and female mice was similar to that of the controls. The mean weights and final mean body weights of exposed male and female mice were similar to those of the controls. There were no clinical findings associated with exposure to talc aerosols.

There were no significant changes in any organ-weight-to-body-weight ratios in exposed male or female mice. Talc lung burdens increased with talc exposure level. However, the ratio of lung burden to exposure concentration was constant at all exposure levels. In contrast to rats, the maximum ability of the respiratory tract to clear particles was apparently not exceeded at the 18 mg/m^3 level.

The only lesions related to inhalation of talc aerosols were observed in the lung of male and female mice exposed to 18 mg/m^3. However, the changes were minimal and consisted of a diffuse increase in the number of intra-alveolar macrophages. In most cases, pulmonary macrophages did not exceed two per alveolus, but occasional clusters of up to 10 alveolar macrophages were observed. The individual macrophages were two to three times normal size with foamy granular cytoplasm.